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(54) METHOD FOR THE EVALUATION OF IMPLANTABLE MATERIALS

VERFAHREN ZUR EVALUIERUNG VON INPLANTIERBAREN MATERIALIEN

TECHNIQUE D'EVALUATION DESTINEE AUX MATERIAUX IMPLANTABLES

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Description

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application claims the benefit of United States Patent Application 60/178,538, entitled "Method For The Evaluation of The Sealing Ability of Dental Products," filed January 26, 2000.

BACKGROUND

[0002] A wide variety of natural and artificial materials are implanted in humans and animals during the treatment of injuries, conditions and diseases. Among the common uses for these materials are as sutures and as filling material for dental cavities.

[0003] A variety of methods are currently used to determine whether materials can prevent bacterial contamination from passing through or around the material, as for example described in Behrend G.D. et al. "An in-vitro study of smear layer removal and microbial leakage along root-canal fillings" International Endodontic Journal. England Mar 1996, vol. 29, no.2, Marh 1996 (1996-03), pages 99-107 ; Ricci G.et al. "In vitro permeability evaluation and colonization of membranes for periodontal regeneration by Porphyromonas gingivalis" Journal of Periodontology, vol. 67, no. 5, 1996, pages 490-496; Peters L.B. et al. "Penetration of bacteria in bovine root dentine in vitro" International Endodontic Journal. England Jan 2000, vol.33, no. 1, January 2000 (2000-1), pages 28-36; EP 0045137 published on 3 February 1982.

[0004] In one method, materials intended to fill cavities in teeth are tested by cleaning out the canals of a natural extracted tooth, sealing the root end of the tooth with the material being tested and filling the center with a test substance. The test substance can be a radioisotope, a dye or bacteria. The sealed tooth is then placed in a container with the sealed end contacting a test medium. Over time, the test medium is checked for presence of the test substance to determine whether the material has effectively prevented the test substance from leaking out of the center of the tooth.

[0005] Though useful, these evaluation methods has several disadvantages. Radioisotope are difficult to work with and are potentially dangerous. The presence of dye in the test medium does not necessarily indicate that bacteria would breech the test material because dyes have a much smaller molecular size than bacteria. Finally, the presence of bacteria in the test medium can indicate that the testing apparatus itself was contaminated rather than that the material was breeched.

[0006] It is further known the use of bacteria modified with light emitting markers in order to perform bioluminescence-based assays as described in Billard P. et al. "Bioluminescence-based assays for detection and characterization of bacteria and chemicals in clinical laboratories" Clinical Bochemistry, Pergamon Press; XX; vol. 31, no.1, February 1998 (1998-01), pages 1-14.

[0007] Additionally, wound closure materials are currently tested by looking at the amount of inflamation the material causes in vivo. However, there is no current method for determining whether wound closure material is subject to bacterial contamination or colonization.

[0008] Therefore, it would be useful to have a method of testing materials to determine whether they are subject to bacterial contamination or colonization. Further, it would be useful to have another method of testing whether materials can prevent bacteria from passing through or around the material.

SUMMARY

[0009] According to one embodiment, the present invention is a method for evaluating whether a material will allow bacteria to pass through the material or pass into the material. The method comprises, first, providing bacteria which are modified to produce a first detectable signal which is light emission in the visible specturm. Then, the bacteria are placed on a first side of the material being evaluated, and a determination is made whether the first signal is present on a second side of the material or within the material. Absence of the first signal on the second side of the material or within the material indicates that the bacteria have not passed through or around the material. Presence of the first signal on the second side of the material or within the material indicates that the bacteria have passed through or around the material.

[0010] In a preferred embodiment, the method additionally comprises quantifying the amount of bacteria that will pass through the material or into the material by quantifying the amount of the first signal on the second side of the material. Increasing amounts of the first signal on the second side of the material or within the material indicates increasing amounts of bacteria that will pass through, around or into the material.

[0011] In another preferred embodiment, the bacteria are modified to produce a second detectable signal, and the method additionally comprises determining whether the second signal is present on the second side of the material or within the material. Absence of the second signal on the second side of the material or within the material indicates that the bacteria have not passed through or around the material or into the material. Presence of the second signal on the second side of the material or within the material indicates that the bacteria have passed through, around or into the material.

[0012] In another preferred embodiment, the second signal is light emission in the visible spectrum. In a particularly preferred embodiment, there the bacteria are modified to incorporate a functional green fluorescent protein. In another particularly preferred embodiment, the bacteria are modified to incorporate a functional luciferase. In yet another particularly preferred embodiment, the bacteria are modified to incorporate both a

functional green fluorescent protein and a functional luciferase.

[0013] In one embodiment of the present invention, placing the bacteria on a first side of the material being evaluated comprises placing the bacteria in the center of a hollowed out, extracted natural tooth where the root end of the tooth is sealed with the material, and then placing the root end of the tooth in a test medium. Then, a determination is made whether the first signal is present on a second side of the material or within the material by detecting the first signal in the test medium or within the material. In a particularly preferred embodiment, the bacteria provided are additionally modified to be grown selectively, such as due to antibiotic resistance.

[0014] According to another embodiment of the present invention, there is provided a method for the evaluation of a material to determine whether the material is susceptible to bacterial contamination or colonization when implanted into an animal or human. The method comprises providing bacteria which are modified to produce a first detectable signal. Next, the material being evaluated is exposed to the bacteria. Then, a determination is made whether the first signal is present on the material or within the material. Absence of the first signal on the material or within the material indicates that the material is not susceptible to bacterial contamination or colonization. Presence of the first signal on the material or within the material indicates that the material is susceptible to bacterial contamination or colonization.

[0015] In a preferred embodiment, the method additionally comprises quantifying the susceptibility of the material to bacterial contamination or colonization by quantifying the amount of the first signal on the material or within the material. Increasing amounts of the first signal on the material or within the material indicates increasing susceptibility of the material to bacterial contamination or colonization.

[0016] In a preferred embodiment, the bacteria are modified to produce a second detectable signal, and the method additionally comprises determining whether the second signal is present on the material or within the material. Absence of the second signal on the material or within the material indicates that the material is not susceptible to bacterial contamination or colonization. Presence of the second signal on the material or within the material indicates that the material is susceptible to bacterial contamination or colonization.

FIGURES

[0017] The features, aspects and advantages of the present invention will become better understood with regard to the following description, appended claims and accompanying figures where:

Figure 1 is a diagram showing the plasmid pXylAdual; and

Figure 2 is a diagram of an apparatus used for testing materials to determine whether the material will allow bacteria to pass through according to the present invention.

DESCRIPTION

[0018] The present method allows the testing of materials for implantation to determine whether they can prevent bacteria from passing through or around the material. Additionally, the present method allows the testing of materials for implantation to determine whether they are susceptible to bacterial contamination or colonization. The present method can be used to evaluate dental materials to be used for restorations, endodontic treatment and the surgical repair of teeth, as well as to evaluate wound closure material. However, the present method can also be used to evaluate other materials for implantation into animals or humans, as will be understood by those with skill in the art with reference to this disclosure.

[0019] As used in this disclosure, the phrase "passing through or around the material" and equivalent phrases means passing into the material, passing through the material from a first side to a second side and passing between the implanted material and the natural part of the animal or human body at the site of implantation from one side of the material to another. For example, when the implantable material is used as filling material for a tooth cavity, the present method allows the testing of the material to see if bacteria will colonize the material itself on the surface, pass into the material, pass entirely through the filling or pass between the edges of the filling where is forms a seal with the remainder of the natural tooth.

[0020] The present method involves the use of modified bacteria. Preferably, the modified bacteria produce a detectable signal when they are living that distinguishes the bacteria from naturally occurring bacteria which might contaminate the apparatuses used in the method. The signal is light emission in the visible spectrum. In a preferred embodiment, the modified bacteria produce a plurality of such detectable signals when they are living.

[0021] In one embodiment, the bacteria are modified to incorporate the cDNA for a functional green fluorescent protein. In another embodiment, the bacteria are modified to incorporate the cDNA for a functional luciferase. In a particularly preferred embodiment, the bacteria are modified to incorporate both the cDNA for a functional green fluorescent protein and the cDNA for a functional luciferase.

[0022] One suitable form of cDNA codes for the green fluorescent protein from the jellyfish *Aequorea victoria.* This form of green fluorescent protein emits green light by accepting energy transfer from sources that include exogenous blue light and from some luciferase catalyzed reactions. The UV light stimulated green fluores-

cent protein fluorescence does not require cofactors and the gene product alone can be sufficient to allow detection of single living cells under the light microscope. However, cDNA's coding for other green fluorescent proteins are also suitable, including modified forms of green fluorescent proteins.

[0023] Another suitable form of cDNA codes for a luciferase from *Xenorhabdus luminescens.* However, cDNA's coding for other luciferases are also suitable including modified forms of luciferases.

[0024] The method for evaluating whether a material will allow bacteria to pass through or around the material is performed as follows. First, bacteria are provided which have been modified to produce a first detectable signal. The bacteria are placed on a first side of the material being evaluated. Then, the bacteria are left in contact with the material for a period of time ranging from about a few minutes to about several months or more. Next, the presence or absence of the first signal is determined on a second side of the material or within the material. The absence of the first signal on the second side of the material or within the material indicates that the bacteria have not passed through or around the material. The presence of the first signal on the second side of the material or within the material indicates that the bacteria have passed through or around the material.

[0025] The method can additionally comprise quantifying the amount of bacteria that will pass through the material by quantifying the amount of the first signal on the second side of the material. Increasing amounts of the first signal on the second side of the material or within the material indicates increasing amounts of bacteria have passed through or around the material.

[0026] Further, the bacteria provided can have been modified to produce a second detectable signal. The method can then additionally comprise determining whether the second signal is present on the second side of the material or within the material. The absence of the second signal on the second side of the material or within the material additionally indicates that the bacteria have not passed through or around the material. The presence of the second signal on the second side of the material or within the material additionally indicates that the bacteria have passed through or around the material. The second signal can be used to confirm the results determined by detecting the first signal on the second side. Further, depending on the signals used, the second signal can add specificity to quantification of the amount of bacteria that have passed through or around the material.

[0027] In one embodiment, the method comprises placing the modified bacteria in the center of a hollowed out, extracted natural tooth. The root end of the tooth is then sealed with the material. The sealed tooth is placed in a test medium with the sealed end covered by the test medium. After a suitable period of time, a determination is made whether the first signal is present on a second side of the material by detecting the first signal in the test medium. The absence of the first signal on the second side of the material or within the material indicates that the bacteria have not passed through or around the material. The presence of the first signal on the second side of the material or within the material indicates that the bacteria have passed through or around the material. The test medium can be a suitable bacteria culture medium to aid in detection of bacteria that have passed through or around the material by allowing bacterial growth and reproduction.

[0028] Similarly, when the bacteria have been modified to produce a second detectable signal, the second signal can also be detected in the test medium when they are living. The absence of the second signal on the second side of the material or within the material indicates that the bacteria have not passed through or around the material. The presence of the second signal on the second side of the material or within the material indicates that the bacteria have passed through or around the material.

[0029] In another preferred embodiment, there the modified bacteria are additionally modified to be grown selectively. One suitable type of selective growth is to modify the bacteria to be antibiotic resistant, though other types of selective growth are possible as will be understood by those with skill in the art with reference to this disclosure. When the bacteria have been modified to be antibiotic resistant, a suitable antibiotic can be included on the second side of the material being evaluated to discourage bacterial growth from contamination, rather than from the modified bacteria passing through the material being evaluated.

[0030] In a preferred embodiment, the present method is a method to determine whether a material is susceptible to bacterial contamination or colonization. The method comprises providing bacteria which are modified to produce a first detectable signal. Next, the material being evaluated is exposed to the bacteria. Then, a determination is made whether the first signal is present on the material. The absence of the first signal on the material indicates that the material is not susceptible to bacterial contamination or colonization. The presence of the first signal on the material indicates that the material is susceptible to bacterial contamination or colonization.

[0031] The method can additionally comprise quantifying the susceptibility of the material to bacterial contamination or colonization by quantifying the amount of the first signal on the material. Increasing amounts of the first signal on the material indicates increasing susceptibility of the material to bacterial contamination or colonization.

[0032] In another preferred embodiment, the bacteria are modified to produce a second detectable signal. The method additionally comprises determining whether the second signal is present on the material or within the material after exposing the material to the bacteria. The absence of the second signal on the material or within

the material indicates that the material is not susceptible to bacterial contamination or colonization. The presence of the second signal on the material or within the material indicates that the material is susceptible to bacterial contamination or colonization.

[0033] Examples of the present method will now be described in greater detail. Modified bacteria containing genes to produce a functional green fluorescent protein, a functional luciferase and to contain an antibiotic resistance gene were constructed for use in the method by transformation with a plasmid DNA bearing a cassette with genes producing luciferase, green fluorescent protein and antibiotic resistance as follows. Two constructs were used. The first construct pLITE201 (as disclosed in Voisey CR, Marincs F. Biotechniques 1998;24:56) was a plasmid vector with a gram-negative origin of replication containing the *lux CDABE* cassette from *Xenorhabdus luminescens* driven by the lac promoter. It was purified from DH5α using the Maxi-Prep DNA purification kit (Qiagen GmbH, Santa Clarita, CA, US). The pLITE201 plasmid was then electroporated into attenuated strains of *Vibrio cholera, Salmonella typhimurium,* and *Shigella* using BioRad® electroporation protocols for the various strains and the BioRad® Gene Pulser II unit (Bio-Rad Laboratories, Hercules, CA). Positive transformants were identified by placing the outgrowth plates under the Argus 100 low light imager (Hamamatsu Corp., Hamamatsu, Japan). The positive colonies were confirmed by observing fluorescent bacteria under the fluorescent microscope.

[0034] The second construct was a *lux ABCDE* cassette from pXylA-dual (Hill, P, University of Nottingham, UK) as shown in Figure 1, purified using the Maxi-Prep kit (Qiagen). This plasmid has a gram-positive origin of replication as well as gram-positive ribosomal binding sites, which allowed expression in gram-positive organisms. The plasmid was then transformed into *Enterococcus faecalis* (strains JH2-2, ATCC4082 , and OG1X) using electroporation with the BioRad® Gene Pulser II (Bio-Rad Laboratories, Hercules, CA).

[0035] Transformation was accomplished as follows. First, a pre-culture of the *E. faecalis* strains was used to inoculate 15 ml of BYGT broth containing 0.7% glycine to weaken the cell walls. The concentration of glycine was determined as that necessary to reduce bacterial growth as determined by the optical density at 600 or 600 nm by about 70 % to 90%. Next, the overnight culture was diluted into pre-warmed BYGT broth containing 0.7% glycine to bring the OD(600) to 0.06-0.08 and the cells were incubated in 37°C without agitation for 1 hour. Then, the cells were chilled on ice and harvested by centrifugation.

[0036] Next, the cells were washed twice with electroporation buffer (0.625 M sucrose, 1 mM $MgCl_2$, pH 4) and the cells were aliquoted into 100:1 volumes and incubated on ice for 30 minutes or deep frozen for later use. Approximately 300 ng of DNA was added and the cells were electroporated using 0.2 cm cuvettes, field strength 6,250 V/cm, resistance 200 Ω, and 25 μF capacitance. The cells were placed on ice for about 1-2 minutes and were diluted into 1 ml THB medium plus antibiotics (Chloramphenicol) and then, incubated at 37°C for 90-120 minutes. Next, the cells were plated on THB agar with 0.25 M sucrose and antibiotics. Colonies were observed in 48 hours under the low light imager (Hamamatsu) and the presence of the plasmid was confirmed by observation of fluorescent bacteria under the fluorescent microscope.

[0037] The method of testing materials for implantation to determine whether they can prevent bacteria from passing through the material according to the present invention was performed as follows. Extracted natural teeth were placed in bleach for 24 hours to remove the organic debris from the external surfaces. Some of the material being tested was tested as dental restorations placed in the coronal aspect of the teeth. Preparations for these tests were made according to the standard protocols for amalgam, composite and crown restorations. The preparations were filled by the test material. Each tooth was placed in a container having a suitable growth medium, antibiotic and the modified bacteria and left for about 48 hours. Then the teeth were removed, sectioned and examined to determine if the bacteria penetrated the material. Luminescence was confirmed by examination under a low light imager.

[0038] The second application tested was as root repair material. The root canal system was cleaned out to leave a hollow center space in the teeth. The root-end was resected 3 mm from the apex with a high speed, hand drill and a fissure burr, and the root-end was prepared to receive a retrofilling material with a high speed, hand drill and a #2 round burr. The test material was used to seal the root end.

[0039] Referring now to Figure 2, there is shown a diagram of an apparatus 10 used for testing materials 12 to determine whether the material will allow bacteria to pass through according to the present invention. Each tooth 14, prepared as described above, was fitted into a microcentrifuge tube 16 and sealed into place using sticky wax so that the root apex 18 was protruding from the tube 16 and fit onto a lower compartment 20 of an apparatus 10. The lower compartment 20 contained liquid broth media and antibiotic or contained solid media and antibiotic, and each tooth was placed into the media. The media and antibiotic were selected based on the strain and antibiotic resistance gene being used. The lid of the microcentrifuge tube was then opened and each tooth 14 was filled with the labeled bacteria in the same liquid broth media containing the antibiotic.

[0040] Leakage of bacteria into the media in the lower chamber 20 was evaluated by placing the media in the chamber under a low light imager, or in a luminometer if liquid media was used, (not shown) to measure the presence or absence of labeled bacteria. Leakage was found when using some materials indicating that the material did not prevent passage of bacteria. Therefore,

this method can be used to determine whether a material can prevent bacteria from passing through the material.

[0041] The method of testing materials for implantation to determine whether they are susceptible to bacterial contamination or colonization according to the present invention was performed as follows. First, an incision was made in the animal skin and the incision was closed by the material being tested in the form of sutures. Approximately 10^7 modified bacterial were intravenously injected into the animal through the femoral vein or through the tail vein. The animals were monitored daily to determine if the modified bacteria were present on the material being tested as indicated by the presence of luminescence at the incision wound under the low light imager. For some materials, no luminescence was present on the material. For other materials, varying amounts of luminescence was present. Therefore, this method can be used to predict whether a material is susceptible to bacterial contamination or colonization when implanted into an animal or human.

[0042] Although the present invention has been discussed in considerable detail with reference to certain preferred embodiments, these embodiments are merely illustrative.

Claims

1. A method for evaluating whether a material will allow bacteria to pass through the material or around the material or into the material comprising:

   a) providing bacteria which are modified to produce a first detectable signal;
   b) placing the bacteria on a first side of the material being evaluated; and
   c) determining whether the first signal is present on a second side of the material or within the material;

   where absence of the first signal on the second side of the material or within the material indicates that the bacteria have not passed through or around the material and where presence of the first signal on the second side of the material or within the material indicates that the bacteria have passed through or around the material; and

   where the first signal is light emission in the visible spectrum..

2. The method according to claim 1 **characterized in that** said bacteria are modified to incorporate one or more than one material selected from the group consisting of a functional green fluorescent protein and a functional luciferase.

3. The method of claim 1 or 2, additionally comprising quantifying the amount of bacteria that will pass through the material or into the material by quantifying the amount of the first signal on the second side of the material;

   where increasing amounts of the first signal on the second side of the material or within the material indicates increasing amounts of bacteria that will pass through the material or into the material.

4. The method according to any one of claims 1 to 3, where placing the bacteria on a first side of the material being evaluated comprises placing the bacteria in the center of a hollowed out, extracted natural tooth where the root end of the tooth is sealed with the material, and then placing the root end of the tooth in a test medium; and

   where determining whether the first signal is present on a second side of the material or within the material comprises detecting the first signal in the test medium or within the material.

5. A method for the evaluation of a material to determine whether the material is susceptible to bacterial contamination or colonization when implanted into an animal or human comprising:

   a) providing bacteria which are modified to produce a first detectable signal;
   b) exposing the material being evaluated to the bacteria; and
   c) determining whether the first signal is present on the material or within the material;

   where absence of the first signal on the material or within the material indicates that the material is not susceptible to bacterial contamination or colonization and where presence of the first signal on the material or within the material indicates that the material is susceptible to bacterial contamination or colonization; and

   where the first signal is light emission in the visible spectrum.

6. The method according to claim 5 **characterized in that** said bacteria are modified to incorporate one or more than one material selected from the group consisting of a functional green fluorescent protein and a functional luciferase.

7. The method of claim 5 or 6, additionally comprising quantifying the susceptibility of the material to bacterial contamination or colonization by quantifying the amount of the first signal on the material or within the material;

   where increasing amounts of the first signal on the material or within the material indicates increasing susceptibility of the material to bacterial contamination or colonization.

8. The method according to any one of claims 1 to 7, where the bacteria provided are additionally modified to be grown selectively.

9. The method of claim 8, where the bacteria grow selectively due to antibiotic resistance.

10. The method according to any one of claims 1 to 9, where the bacteria are modified to produce a second detectable signal, and where the method additionally comprises determining whether the second signal is present on the material or within the material;

where absence of the second signal on the material or within the material indicates that the material is not susceptible to bacterial contamination or colonization and where presence of the second signal on the material or within the material indicates that the material is susceptible to bacterial contamination or colonization.

11. The method of claim 10, where placing the bacteria on a first side of the material being evaluated comprises placing the bacteria in the center of a hollowed out, extracted natural tooth where the root end of the tooth is sealed with the material, and then placing the root end of the tooth in a test medium; and

where determining whether the second signal is present on a second side of the material or within the material comprises detecting the second signal in the test medium or within the material.

12. The method of claim 10 or 11, where the second signal is light emission in the visible spectrum.

Patentansprüche

1. Verfahren zum Evaluierung, ob ein Material erlaubt, dass Bakterien durch das Material hindurch oder um das Material herum oder in das Material eindringen, umfassend:

a) Bereitstellen von Bakterien, die modifiziert sind, um ein erstes nachweisbares Signal zu produzieren;

b) Platzieren der Bakterien auf einer ersten Seite des evaluierten Materials; und

c) Bestimmen, ob das erste Signal auf einer zweiten Seite des Materials oder in dem Material vorhanden ist;

wobei das Nichtvorhandensein des ersten Signals auf der zweiten Seite des Materials oder in dem Material anzeigt, dass die Bakterien nicht durch das Material hindurch oder um das Material herum eingedrungen sind, und wobei das Vorhandensein des ersten Signals auf der zweiten Seite des Materials oder in dem Material anzeigt, dass die Bakterien durch das Material hindurch oder um das Material herum eingedrungen sind; und

wobei das erste Signal die Emission von Licht im sichtbaren Spektrum ist.

2. Verfahren nach Anspruch 1, **dadurch gekennzeichnet, dass** die Bakterien modifiziert sind, um ein oder mehr als ein Material aufzunehmen, das aus der Gruppe bestehend aus einem funktionellen grün fluoreszierenden Protein und einer funktionellen Luziferase ausgewähit ist.

3. Verfahren nach Anspruch 1 oder 2, des Weiteren umfassend das Quantifizieren der Menge an Bakterien, die durch das Material hindurch oder in das Material eindringen, indem die Menge des ersten Signals auf der zweiten Seite des Materials quantifiziert wird;

wobei steigende Mengen des ersten Signals auf der zweiten Seite des Materials oder in dem Material steigende Mengen von Bakterien anzeigen, die durch das Material hindurch oder in das Material eindringen.

4. Verfahren nach einem der Ansprüche 1 bis 3, wobei das Platzieren der Bakterien auf einer ersten Seite des evaluierten Materials das Platzieren der Bakterien in der Mitte eines ausgehöhlten, extrahierten, natürlichen Zahns umfasst, wobei das Wurzelende des Zahns mit dem Material versiegelt wird und dann das Wurzetende des Zahns in ein Testmedium gegeben wird; und

wobei der Nachweis, ob das erste Signal auf einer zweiten Seite des Materials oder in dem Material vorhanden ist, den Nachweis des ersten Signals in dem Testmedium oder in dem Material umfasst.

5. Verfahren für die Evaluierung eines Materials um zu bestimmen, ob das Material gegenüber bakterieller Kontamination oder Besiedelung anfällig ist, wenn es in ein Tier oder einen Menschen eingepflanzt wird, umfassend:

a) Bereitstellen von Bakterien, die modifiziert sind, um ein erstes nachweisbares Signal zu produzieren;

b) Aussetzen des evaluierten Materials gegenüber den Bakterien; und

c) Bestimmen, ob das erste Material auf dem Material oder in dem Material vorhanden ist;

wobei das Nichtvorhandensein des ersten Signals auf dem Material oder in dem Material anzeigt, dass das Material nicht gegenüber einer bakteriellen Kontamination oder Besiedelung anfällig ist, und wobei das Vorhandensein des ersten Signals auf dem Material oder in dem Material anzeigt, dass das Material gegenüber einer bakteriellen Kontamination oder Besiedelung anfällig ist; und

wobei das erste Signal die Emission von Licht im sichtbaren Spektrum ist.

6. Verfahren nach Anspruch 5, **dadurch gekennzeichnet, dass** die Bakterien modifiziert sind, um ein oder mehr als ein Material, das aus der Gruppe bestehend aus einem funktionellen, grün fluoreszierenden Protein und einer funktionellen Luziferase ausgewählt ist, aufzunehmen.

7. Verfahren nach Anspruch 5 oder 6, des Weiteren umfassend das Quantifizieren der Anfälligkeit des Materials gegenüber bakterieller Kontamination oder Besiedelung, indem die Menge des ersten Signals auf dem Material oder in dem Material quantifiziert wird;

wobei steigende Mengen des ersten Signals auf dem Material oder in dem Material eine erhöhte Anfälligkeit des Materials gegenüber bakterieller Kontamination oder Besiedelung anzeigen.

8. Verfahren nach einem der Ansprüche 1 bis 7, wobei die bereit gestellten Bakterien zusätzlich modifiziert sind, um selektiv gezüchtet werden zu können.

9. Verfahren nach Anspruch 8, wobei die Bakterien aufgrund einer Antibiotika-Resistenz selektiv wachsen.

10. Verfahren nach einem der Ansprüche 1 bis 9, wobei die Bakterien modifiziert sind, um ein zweites, nachweisbares Signal zu produzieren und wobei das Verfahren des Weiteren den Nachweis des Vorhandenseins des zweiten Signals auf dem Material oder in dem Material umfasst;

wobei das Nichtvorhandensein des zweiten Signals auf dem Material oder in dem Material anzeigt, dass das Material nicht gegenüber einer bakteriellen Kontamination oder Besiedelung anfällig ist, und wobei das Vorhandensein des zweiten Signals auf dem Material oder in dem Material anzeigt, dass das Material gegenüber einer bakteriellen Kontamination oder Besiedelung anfällig ist.

11. Verfahren nach Anspruch 10, wobei das Platzieren der Bakterien auf einer ersten Seite des evaluierten Materials das Platzieren der Bakterien in der Mitte eines ausgehöhlten, extrahierten, natDrlichen Zahns umfasst,

wobei das Wurzelende des Zahns mit dem Material versiegelt wird und dann das Wurzelende des Zahns in ein Testmedium gegeben wird; und

wobei der Nachweis, ob das erste Signal auf einer zweiten Seite des Materials oder in dem Material vorhanden ist, den Nachweis des ersten Signals in dem Testmedium oder in dem Material umfasst.

12. Verfahren nach Anspruch 10 oder 11, wobei das zweite Signal die Emission von Licht im sichtbaren Spektrum ist.

Revendications

1. Procédé pour évaluer si des bactéries pourront traverser une matière ou la contourner ou passer dans celle-ci comprenant :

a) l'apport de bactéries qui sont modifiées pour produire un premier signal détectable ;
b) la mise en place des bactéries sur un premier côté de la matière en cours d'évaluation ; et
c) le fait de déterminer si le premier signal est présent sur le deuxième côté de la matière ou dans celle-ci ;

dans lequel l'absence du premier signal sur le deuxième côté de la matière ou dans celle-ci indique que les bactéries n'ont pas traversé ou n'ont pas contourné la matière et dans lequel la présence du premier signal sur le deuxième côté de la matière ou dans celle-ci indique que les bactéries ont traversé ou ont contourné la matière ; et

dans lequel le premier signal est une émission luminescente dans le spectre visible.

2. Procédé selon la revendication 1, **caractérisé en ce que** lesdites bactéries sont modifiées pour incorporer une ou plus d'une matière choisie dans le groupe comprenant une protéine verte fluorescente fonctionnelle et une luciférase fonctionnelle.

3. Procédé selon la revendication 1 ou la revendication 2, comprenant en outre le chiffrage de la quantité de bactéries qui traverseront la matière ou la contourneront en quantifiant la quantité du premier signal sur le deuxième côté de la matière ;

dans lequel des quantités de plus en plus importantes du premier signal sur le deuxième côté de la matière ou dans la matière indiquent des quantités de plus en plus importantes de bactéries qui traverseront ou contourneront la matière.

4. Procédé selon l'une quelconque des revendications 1 à 3, dans lequel la mise en place des bactéries sur un premier côté de la matière en cours d'éva-

luation comprend la mise en place des bactéries au centre d'une dent naturelle évidée, extraite où l'extrémité de la racine de la dent est obturée avec la matière, et ensuite la mise en place de l'extrémité de la racine de la dent dans un milieu d'essai ; et

dans lequel le fait de déterminer si le premier signal est présent sur le deuxième côté de la matière ou dans celle-ci comprend le dépistage du premier signal dans le milieu d'essai ou dans la matière.

5. Procédé d'évaluation d'une matière pour déterminer si la matière est prédisposée à une contamination ou une multiplication bactérienne lorsqu'elle est implantée chez un animal ou un être humain comprenant :

a) l'apport de bactéries qui sont modifiées pour produire un premier signal détectable ;
b) l'exposition de la matière en cours d'évaluation aux bactéries ; et
c) le fait de déterminer si le premier signal est présent sur la matière ou dans celle-ci ;

dans lequel l'absence du premier signal sur la matière ou dans celle-ci indique que la matière n'est pas prédisposée à une contamination ou une multiplication bactérienne et dans lequel la présence du premier signal sur la matière ou dans celle-ci indique que la matière est prédisposée à une contamination ou une multiplication bactérienne ; et

dans lequel le premier signal est une émission luminescente dans le spectre visible.

6. Procédé selon la revendication 5, **caractérisé en ce que** lesdites bactéries sont modifiées pour incorporer une ou plus d'une matière choisie dans le groupe comprenant une protéine verte fluorescente fonctionnelle et une luciférase fonctionnelle.

7. Procédé selon la revendication 5 ou la revendication 6, comprenant en outre le chiffrage de la prédisposition de la matière à la contamination ou à la multiplication bactérienne en quantifiant la quantité du premier signal sur la matière ou dans la matière ;

dans lequel des quantités de plus en plus importantes du premier signal sur la matière ou dans la matière indiquent une prédisposition de plus en plus importante de la matière à la contamination ou à la multiplication bactérienne.

8. Procédé selon les revendications 1 à 7, dans lequel les bactéries fournies sont en outre modifiées pour être cultivées de façon sélective.

9. Procédé selon la revendication 8, dans lequel les bactéries sont cultivées de façon sélective en raison d'une résistance aux antibiotiques.

10. Procédé selon l'une quelconque des revendications 1 à 9, dans lequel les bactéries sont modifiées pour produire un deuxième signal détectable, et dans lequel le procédé comprend en outre le fait de déterminer si le deuxième signal est présent sur la matière ou dans celle-ci ;

dans lequel l'absence du deuxième signal sur la matière ou dans celle-ci indique que la matière n'est pas prédisposée à une contamination ou une multiplication bactérienne et dans lequel la présence du deuxième signal sur la matière ou dans celle-ci indique que la matière est prédisposée à une contamination ou une multiplication bactérienne.

11. Procédé selon la revendication 10, dans lequel la mise en place des bactéries sur un premier côté de la matière en cours d'évaluation comprend la mise en place des bactéries au centre d'une dent naturelle évidée, extraite où l'extrémité de la racine de la dent est obturée avec la matière, et ensuite la mise en place de l'extrémité de la racine de la dent dans un milieu d'essai ; et

dans lequel le fait de déterminer si le deuxième signal est présent sur le deuxième côté de la matière ou dans celle-ci comprend la détection du deuxième signal dans le milieu d'essai ou dans la matière.

12. Procédé selon la revendication 10 ou la revendication 11, dans lequel le deuxième signal est une émission luminescente dans le spectre visible.

FIG. 1

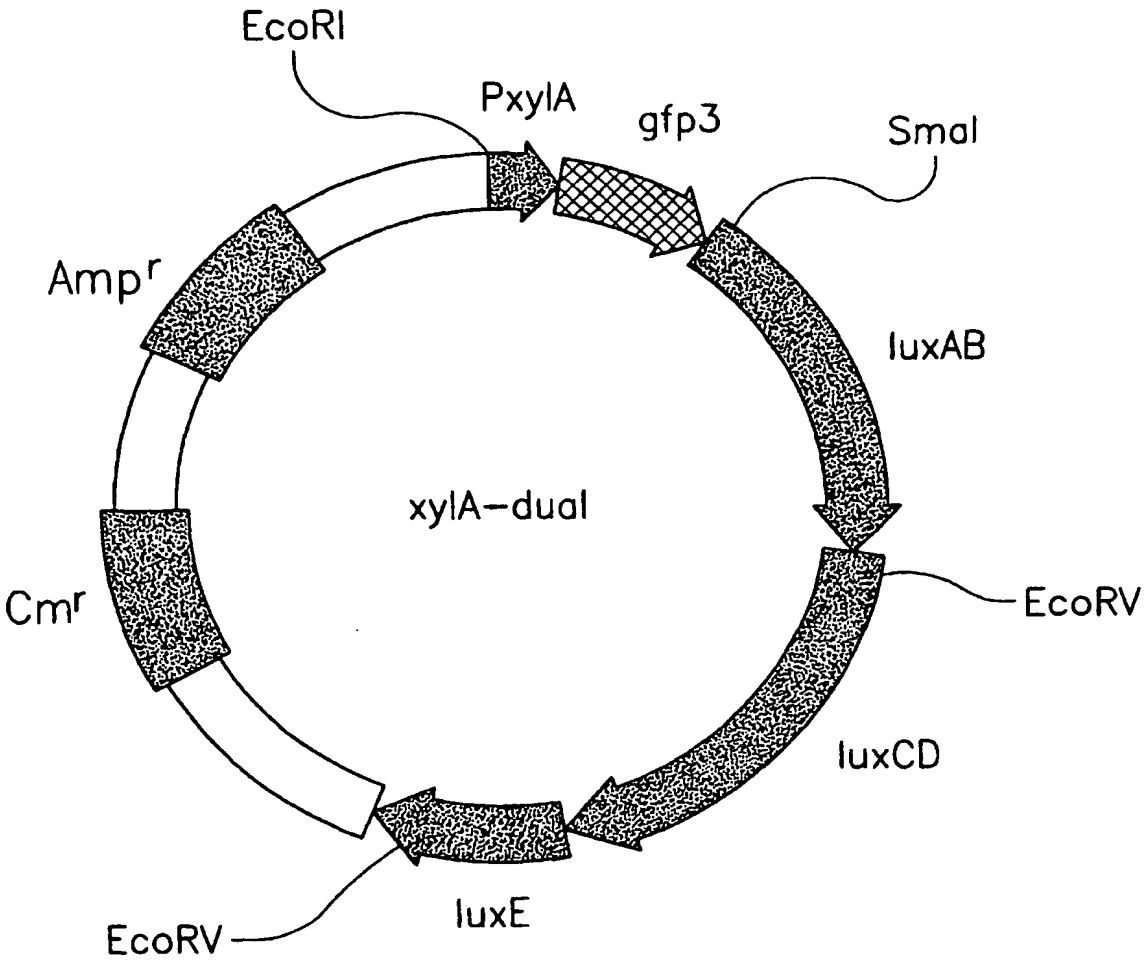
EcoRI
PxylA
gfp3
SmaI
Amp^r
luxAB
xylA–dual
Cm^r
EcoRV
luxCD
EcoRV
luxE

FIG. 2